the surrounding fluid, we use fluorescence to measure the local temperature in the nanometer space around these nanoparticles. We compare isolated nanoparticles to particles arranged on a surface or in bulk, while also recording the global temperature. The obtained spatio-temporal temperature profiles are modeled using finite element software (COMSOL).

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Temperature-Mediated Effects of Millimeter Wave Stimulation on Membrane Protein Function

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Despite numerous demonstrations that millimeter waves (MMW) induce changes in cellular activity in neuronal and muscle preparations, the mechanisms underlying these changes remain unclear. Given the high aqueous absorbance at millimeter wavelengths, thermal mechanisms are likely. However, non-thermal mechanisms based on resonant effects have also been postulated. We examined the effects of MMW stimulation in a simplified preparation comprising *Xenopus laevis* oocytes expressing canonical ion channels and transporters that underlie membrane excitability. Oocytes were injected with RNA encoding the *Drosophila* voltage-gated potassium channel Shaker, the rat voltage-gated sodium channel $Na_V1.4$ with its β1 subunit, a mixture of the α and β1 subunits of the squid sodium-potassium pump, or an "AP mix" of the Shaker and $Na_V1.4$ channels enabling action potential (AP) generation. Electrophysiological responses to MMW were studied using two electrode voltage and current clamps. In this system, MMW (0 to 170 mW/cm^2 at 60 GHz) applied directly to oocytes via a home-built waveguide setup produced local temperature increases of 0.5 to 5° C on a timescale of 1-10 seconds. MMW radiation altered Shaker activation kinetics and voltage dependence and $Na_V1.4$ inactivation kinetics and voltage dependence. MMW also accelerated the activity of the sodium-potassium pump. In AP mix-injected oocytes, the AP firing rate under current clamp increased with the increasing power of applied MMW, and higher power led to truncated AP trains. The observed effects of MMW on ion channels and transporters are consistent with purely thermal effects. Likewise, changes in AP firing are predicted by thermal dependencies in the Hodgkin and Huxley model. Our results suggest that MMW stimulation produces significant thermally-mediated effects on excitable cells that must be taken into account in the study and use of this range of wavelengths in biology and medicine.

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Light-Controlled Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway in Live Cells

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The Mitogen-Activated Protein Kinase (MAPK) signaling pathway regulates critical cellular function such as cell proliferation, differentiation, and apoptosis. Defective MAPK signaling has been frequently discovered in various cancers such as in breast cancer, lung cancer, and melanoma. Evidence showed that signaling output of the MAPK pathway depends critically on its spatiotemporal regulation. However, there are very limited means to control its spatial and temporal dimension in live cells with high accuracy. Here, we report a light-gated protein-protein interaction system that precisely regulates the activation and inactivation of the MAPK signaling pathway. We show that sustained MAPK activation through continuous light stimulation is sufficient to induce significant neurite outgrowth in PC12 cells in the absence of nerve growth factor. Light-gated activation leads to an interesting discovery that MAPK alone is sufficient to account for neurite elongation, it only partially contributes to the full development of sodium channels in PC12 cells. The strategy of using light-gated protein interaction shows a great promise in dissecting detailed mechanisms of signal transduction in cells.

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Remote Control of Myosin Speed and Directionality using Light-Activated Gear-Shifting

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Engineering molecular motors with dynamically controllable properties will allow selective perturbation of mechanical processes in vivo and provide sophisticated components for directed nanoscale transport in vitro. We previously constructed myosin motors that respond to a change in [Ca^{++}] by reversing their direction of motion along the polarized actin filament [1]. To expand the potential applications of controllable molecular motors, we have now developed myosins that shift gears in response to blue light illumination. Light is a versatile control signal that can be readily modulated in time and space, and is generally orthogonal to cellular signaling. Using structure-guided protein engineering, we have incorporated LOV photoreceptor domains into the lever arms of chimeric myosins, resulting in motors that robustly speed up, slow down, or switch directions upon illumination. These genetically encoded motors should be directly deployable inside living cells. Our successful designs include constructs based on two different myosin classes, and we show that optical velocity control can be implemented in motors that move at microns/sec speeds, enabling practical biological and bioengineering applications.

[1] Chen, L., Nakamura, M., Schindler,T.D., and Bryant Z. (2012). Nat. Nanotechnol. 7, 252-6).

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Generation of a Light Inhibited Src Kinase through Insertion of LOV into the Catalytic Domain

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Src is a non-receptor tyrosine kinase that participates in a diverse spectrum of signaling pathways. Because the interactions of Src with activators and downstream ligands depend on the spatio-temporal dynamics of Src activation, it will be valuable to control the timing and location of activation in vivo with light. Through insertion of the light oxygen voltage (LOV) domain into a conserved portion of the kinase catalytic domain, we have generated a genetically encoded analog of Src that is reversibly inhibited upon irradiation at wavelengths between 400 and 500 nm. With optimized linkers, the insertion minimally perturbed Src catalytic activity in the dark, but led to a greater than 2-fold reduction in activity upon irradiation. Molecular dynamics studies indicate that the light-induced unwinding of the LOV Jα-helix results in narrowing of the ATP binding site. The activation loop and beta-sheet move closer to each other, emulating the structure of the inactive state. Photoinhibition of Src reduced fibroblast migration rates and the frequency of protrusion. Cells transformed by overexpression of LOV-Src in the dark could be reverted to a normal phenotype upon irradiation. Structural studies and insertion of other engineered folds into the same Src site indicate that this strategy can be broadly applied to other kinases.

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In Vivo Imaging of Odor-Evoked Responses in the Olfactory Bulb using Arclight, a Novel Fp Voltage Probe

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Optogenetic reporters of membrane potential allow for recording of genetically distinct populations of neurons, although their usefulness to date has been limited by poor in vivo expression, small signal sizes and slow kinetics. The recently developed fluorescent protein (FP) voltage probe ArcLight exhibits a change in fluorescence to a 100 mV depolarization five times larger than previously reported probes in HEK 293 cells. However, recordings of ArcLight in mammalian neurons have been limited to cultured neurons. To demonstrate the utility of this probe in an in vivo preparation, AAV-1 viral transduction was used to express ArcLight in the mouse olfactory bulb. Different odors were presented, and the resulting patterns of activation were imaged and compared with those recorded from the optogenetic calcium indicator GCaMP3. Odor-specific patterns of activation were imaged from both ArcLight and GCaMP3, indicating that the FP voltage sensor ArcLight can be used as a reliable detector of odor-evoked population signals in the mouse olfactory bulb.

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Modification of Arclight, a Genetically-Encoded Voltage Sensitive Probe: A Study of Mechanism

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Arclight is a fusion protein of superEcliptic pHluorin-A227D fluorescent protein and the voltage sensing domain of the Ciona voltage sensitive phosphatase.